## ABSTRACT

Hepatotoxicity and infertility are major concerns all over the world. Liver dysfunction is one of the key causes of death on account of organ failure. About 15% of couples are infertile, among them, 50% are due to male impotence. Chromium (Cr(VI)) is used in industry inevitably, which induces toxicity in animals. The current study aimed to evaluate the ameliorative potential of *Nigella sativa* and *Aloe vera* and their conjugated AgNPs against hexavalent chromium induced hepatotoxicity and testicular toxicity in mice model. The *Nigella sativa* (NS) seed extract and *Aloe vera* (AV) leaf gel extract were used to synthesize bioconjugated silver nanoparticles by using silver nitrate solution. The biofabricated AgNPs formation was confirmed by using a UV visible spectrophotometer (UV-Vis), fourier transforms infrared spectroscopy (FTIR), X-ray diffraction analysis (XRD), and scanning electron microscopy (SEM). The UV Vis spectrum analysis of *Nigella sativa* fabricated AgNPs (NS+NP) and *Aloe vera* fabricated AgNPs (AV+NP) showed an absorption peak at 435 nm and 369 nm, respectively. The SEM analysis of NS+NP and AV+NP showed a spherical shape with some angular morphology. The FTIR analysis of NS+NP and AV+NP showed a band shifting due to Ag+ conjugation and indicated the presence of flavonoids. The XRD analysis of NS+NP and AV+NP showed a crystalline structure of AgNPs.Hexavalent chromium was dissolved in distilled water and orally administered once a day for 60 consecutive days to induce hepatotoxicity and testicular toxicity in mice model. The NS and AV extract and silver nanoparticles, AgNP (chemically synthesized), and standard drug i.e. clomiphene citrate were orally administered to mice for 60 consecutive days in individual and prevention groups. However, in the post treatment groups, the extract and nanoparticles of NS and AV were administered for 30 consecutive days in Cr (VI) intoxicated mice. The ameliorative potential of *Nigella sativa* (NS), *Nigella sativa* mediated silver nanoparticles (NS+NP), *Aloe vera* (AV), and *Aloe vera* mediated silver nanoparticles (AV+NP) were assessed through various biochemical parameters, hematology, computer assisted semen analysis, metal estimation, histology, smear study, and micrometry.

Our outcomes exhibited that oral administration (50 mg/kg BW) of NS, NS+NP, AV, and AV+NP (for 60 and 30 consecutive days) gradually recouped the body weight, and organ indices and ameliorated the histopathological alterations of the liver and testes in Cr induced toxicity in mice.Water intake was higher in Cr treated group however, in all other treatment groups the water intake was not significantly changed. Oral administration of hexavalent chromium (Cr) (1.5 mg/kg BW) for 60 consecutive days induced the highest significant reduction in the antioxidant defense system including catalase (CAT): (87.40±6.01 mmol/mL), superoxide dismutase (SOD): (61.40±2.48 mmol/mL) and glutathione (GSH): (1.54±0.1 µmol/mL).However, oral administration (50 mg/kg BW) of NS, NS+NP, AV, and AV+NP enhanced the level of CAT, SOD, and GSH up to a significant level. The highest significant increase has been shown in alanine aminotransferase (ALT) level (163.0±4.1 U/L) in Cr exposed mice when compared to control group mice (42.4±1.9 U/L), with oral administration (50 mg/kg BW) of NS, NS+NP, AV, and AV+NP exhibited a decline in the level of ALT. The highest significant up-surge has been shown in aspartate aminotransferase (AST) level (484.0±8.1 U/L) in Cr exposed mice when compared to control group mice (84.2±1.5 U/L) whereas oral administration (50 mg/kg BW) of NS, NS+NP, AV, and AV+NP exhibited the highest significant decline in the level of AST. The highest significant elevation has been seen in alkaline phosphatase (ALP) level (337.6±7.2 U/L) in Cr exposed mice whereas, oral administration (50 mg/kg BW) of NS, NS+NP, AV, and AV+NP revealed the highest significant decrease in the level of ALP. Similarly, the highest significant up-surge has been observed in the level of malondialdehyde (MDA) and creatinine in Cr treated mice. However, in treatment groups, significant decline was found in the level of MDA and creatinine. Total protein and albumin levels were measured which were significantly decreased in Cr treated mice (2.9±0.2 g/dL; 2.1±0.2 U/L) which increased up to a significant level with oral intake of *Nigella sativa*, *Nigella sativa* mediated silver nanoparticles, *Aloe vera* and *Aloe vera* mediated silver nanoparticles. Meanwhile, lactate dehydrogenase (LDH) and total bilirubin levels were measured which showed a significant upsurge in Cr treated mice, which down regulated by *Nigella*

*sativa*, *Nigella sativa* mediated silver nanoparticles, *Aloe vera* and *Aloe vera* mediated silver nanoparticles administration upto significant level. During hormone analysis, FSH level was increased (160.0±4.98 ng/mL), whereas LH (1.6±0.1 ng/mL) and testosterone (2.6±0.2 ng/mL) levels were decreased in Cr treated mice. The level of hormones was gradually improved with oral intake of *Nigella sativa*, *Nigella sativa* mediated silver nanoparticles, *Aloe vera*, and *Aloe vera* mediated silver nanoparticles. When the hematological parameters were measured, the upsurge of WBC (6.5±0.4 $10^3/\mu L$) has been found, whereas RBCs (3.0±0.2 $10^6/\mu L$) and platelets (126.6±11.5 $10^3/\mu L$) number was decreased in Cr treated mice. In RBCs, pretreatment, or post treatment groups with NS, NS+NP, AV and AV+NP no significant difference was observed when compared to Cr exposed group. The chromium content was significantly higher in liver, kidney, and testes in Cr treated group. However in treatment groups, Cr content was very low which shows Cr was significantly removed in NS, NS+NP, AV, and AV+NP treatment groups. Computer assisted semen analysis (CASA) showed a decrease in the total number of sperm count, motility, velocities and kinamatics in Cr exposed mice whereas in pre and post treatment groups sperm count, motility, velocities and kinamatics were improved. However, in *Nigella sativa* and *Nigella sativa* mediated AgNP groups results were much better than *Aloe vera* in CASA parameters. The micrometery analysis of the size of hepatocytes (1188.2±467.7 $\mu^2$) and the size of the nucleus of hepatocytes (456.4±206.7 $\mu^2$) showed an upsurge in Cr treated group when compared to the control group (836.8±224.0 $\mu^2$; 254.3±105.5 $\mu^2$ respectively). However, in treatment groups, the size was decreased up to a significant level. The size of the brush border was significantly decreased in Cr exposed mice (10.1±3.0 μ) as compared to the control (20.1±2.6 μ). In testicular micrometric analysis, the cross section area (CSA) of the seminiferous tubule, size of spermatogonia, and spermatocytes were significantly increased in Cr treated group (1094.7±49.8 $mm^2$; 41.3±1.2 $\mu^2$ and 25.1±1.1 $\mu^2$ respectively) when compared to control. However, in treatment groups, the size was decreased up to a significant level. In spermatic cords, the number of spermatogonia and spermatocytes was decreased in Cr treated groups

when compared to the control. However, in treatment groups, the number was improved but not up to a significant level. The micrometric analysis of sperm head breadth, length, middle piece, and tail length was significantly reduced in Cr treated group when compared to the control. However, treatment groups with NS, NS+NP, AV, and AV+NP showed significantly improved parameters of sperm structure in prevention and post treatment groups. In testes and sperm cases, *Nigella sativa* and their mediated AgNP showed much better results in treatment groups than *Aloe vera* and their mediated AgNP. These results demonstrate that *Nigella sativa* and *Aloe vera* extract and their mediated silver nanoparticles have an obvious hepatoprotective and testicular protective activity in Cr (VI) induced hepatotoxicity and testicular toxicity in mice, improving the antioxidant defense and decreasing oxidative stress on intoxicated model mice.